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Teratogenic mechanisms of medical drugs

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TABLE OF CONTENTS

- Introduction
- Methods
- Folate Antagonism
- Neural Crest Cell Disruption
- Endocrine Disruption: Sex Hormones
- Oxidative Stress
- Vascular Disruption
- Specific Receptor- or Enzyme-mediated Teratogenesis
- Summary

BACKGROUND: Although prescription drug use is common during pregnancy, the human teratogenic risks are undetermined for more than 90% of drug treatments approved in the USA during the past decades. A particular birth defect may have its origins through multiple mechanisms and possible exposures, including medications. A specific pathogenic process may result in different outcomes depending upon factors such as embryonic age at which a drug is administered, duration and dose of exposure and genetic susceptibility. This review focuses on the teratogenic mechanisms associated with a number of medications.

METHODS: We used three methods to identify the teratogenic mechanisms of medications: the MEDLINE and EMBASE databases, two recent books on teratogenic agents and a list of drugs classified as U.S. Food and Drug Administration class D or X. Mechanisms were included only if they are associated with major structural birth defects and medications that are used relatively frequently by women of reproductive age.

RESULTS: We identified six teratogenic mechanisms associated with medication use: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis. Many medications classified as class X are associated with at least one of these mechanisms.

CONCLUSIONS: Identifying teratogenic mechanisms may not only be relevant for etiologic and post-marketing research, but may also have implications for drug development and prescribing behavior for women of reproductive age, especially since combinations of seemingly unrelated prescription and over the counter medications may utilize similar teratogenic mechanisms with a resultant increased risk of birth defects.

Key words: congenital abnormalities / pharmaceutical agents / pregnancy / teratology

Introduction

Since approximately half of the pregnancies in the USA are unintended (Finer and Henshaw, 2006), many women expose their unborn children to drugs before they know they are pregnant. Furthermore, prescription drug use is common during pregnancy in many other countries as well, with prevalence estimates ranging from 44 to 79% in several European countries (Olesen *et al.*, 1999; Bakker *et al.*, 2006; Engeland *et al.*, 2008). Because pregnant women were often excluded from clinical trials and data from animal studies are not always predictive for a teratogenic effect in humans, drug use by pregnant women can be considered experimental in most instances. Nevertheless, the use of medication is sometimes inevitable in the treatment of women of reproductive age and during pregnancy. Although it has clearly been shown that some drugs, e.g. thalidomide and isotretinoin, can produce birth defects, the teratogenic risks in human pregnancy are undetermined for more than 90% of drug treatments approved in the USA in the last decades [Lo and Friedman, 2002; Physicians' Desk Reference, 2009 or the website of the Teratogen Information System (TERIS) for more details]. Birth defects are the leading cause of infant mortality and the etiologic pathways are largely unknown for many defects. A particular birth defect may be caused by many different factors (e.g. genetics, environmental agents, medications, physical conditions) as well as by different mechanisms, whereas a specific pathogenic process may result in different outcomes for chemical or drug exposures depending upon such factors as embryonic age, duration and dose of exposure and genetic susceptibility (Pollard, 2007; Schaefer *et al.*, 2007). In addition, maternal determinants, including drug administration, distribution, metabolism, and excretion, may also play an important role. Although the mechanisms by which drugs may cause birth defects are still not completely understood, we will present an overview of the most important teratogenic mechanisms known today. Identifying these mechanisms may be relevant for drug development, (post-marketing) research and prescribing of medications to women in their reproductive years.

Methods

We used three methods to identify the most important teratogenic mechanisms associated with medical drug use. First, in January 2009, the MEDLINE and EMBASE bibliographic databases were used as search engines employing a combination of keywords, including 'birth defects', 'congenital abnormalities', 'mechanism', 'teratogenesis', 'abnormalities, drug-induced', 'pregnancy' and 'pharmaceutical preparation'. Only articles that were published in the English language were included. Secondly, two recent books on teratogenic agents by Shepard and Lemire (2007) and Schaefer *et al.* (2007) were hand-searched for additional mechanisms. Finally, all medications classified by the U.S. Food and Drug Administration (FDA) as class D ('the potential benefits from the use of the drug in pregnant women may be acceptable despite its potential risks') or class X ('contraindicated in women who are or may become pregnant') (U.S. Food and Drug Administration, 2003; Schwarz *et al.*, 2007) were screened. Only mechanisms producing major structural birth defects associated with medications that are relatively frequently used by women of reproductive age (defined as an annual prescription rate of >0.5%, if known) were included in this review. These mechanisms are

oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis. It should be noted that, so far, some of these mechanisms are principally understood from animal models; however, these mechanisms may produce birth defects in humans as well. In addition, some drugs may be involved in multiple mechanisms for producing birth defects.

Folate Antagonism

Folate, the generic term for a water-soluble B vitamin, occurs in high concentrations in certain natural foods (fruits, leafy green vegetables, beans and liver) as polyglutamate. The synthetic form, folic acid (a monoglutamic acid), is used in food fortification and vitamin preparations. Folic acid has a higher bioavailability than food folate (Brouwer *et al.*, 1999). Folate is converted through two reduction reactions by dihydrofolate reductase (DHFR) to the naturally bioactive form tetrahydrofolate (THF), which is converted into 5-methyltetrahydrofolate (5-MTHF) monoglutamate. 5-MTHF is the main form of folate in the blood circulation and is transported into cells by three routes: by membrane-associated receptors, by a carrier-mediated system, the reduced folate carrier, and by passive diffusion (Antony, 1992; van der Put *et al.*, 2001). Inside the cell, it acts as an essential co-enzyme in many biochemical reactions by being an acceptor or donor of one-carbon units in, for example, purine and pyrimidine synthesis and DNA methylation reactions (Fig. 1). Since rapidly proliferating tissues require DNA synthesis the most, it is obvious that folate-dependent reactions are essential for fetal growth and development and that folate requirements increase during pregnancy. In addition, DNA methylation is known to be involved in the epigenetic control of gene expression during development.

Several drugs disturb the folate metabolism and may have a teratogenic effect through inhibition of the folate methylation cycle (Table I). Two general groups of drugs act as folate antagonists. The first group consists of competitive inhibitors of DHFR and includes methotrexate, sulfasalazine, triamterene and trimethoprim, which block the conversion of folate to THF by binding irreversibly to the enzyme (Lambie and Johnson, 1985). They are used in the treatment of a variety of diseases, such as inflammatory bowel disease, rheumatoid arthritis, hypertension and urinary tract infections. The second group of drugs may antagonize other enzymes in the folate metabolism, impair folate absorption or increase folate degradation. This group primarily consists of anti-epileptic drugs, including valproic acid, carbamazepine and phenytoin. The teratogenicity of folate antagonists in humans was first suggested by reports of women who were given aminopterin in the first trimester of pregnancy to induce abortion (Thiersch, 1952). Some anti-epileptic drugs, e.g. carbamazepine and valproic acid, are generally known to increase the risk of folate-sensitive birth defects, such as neural tube defects, orofacial clefts and limb defects. So far, only three studies have been conducted to determine the effect of folate antagonists as a group on the occurrence of birth defects in humans, but the results are inconsistent, particularly for DHFR inhibitors (Hernández-Díaz *et al.*, 2000, 2001; Meijer *et al.*, 2005). In addition, polymorphisms in genes associated with the folate metabolism, including methylenetetrahydrofolate reductase (MTHFR; Botto and Yang, 2000; van Rooij *et al.*, 2003), methionine synthase reductase (MTRR; van der Linden *et al.*,

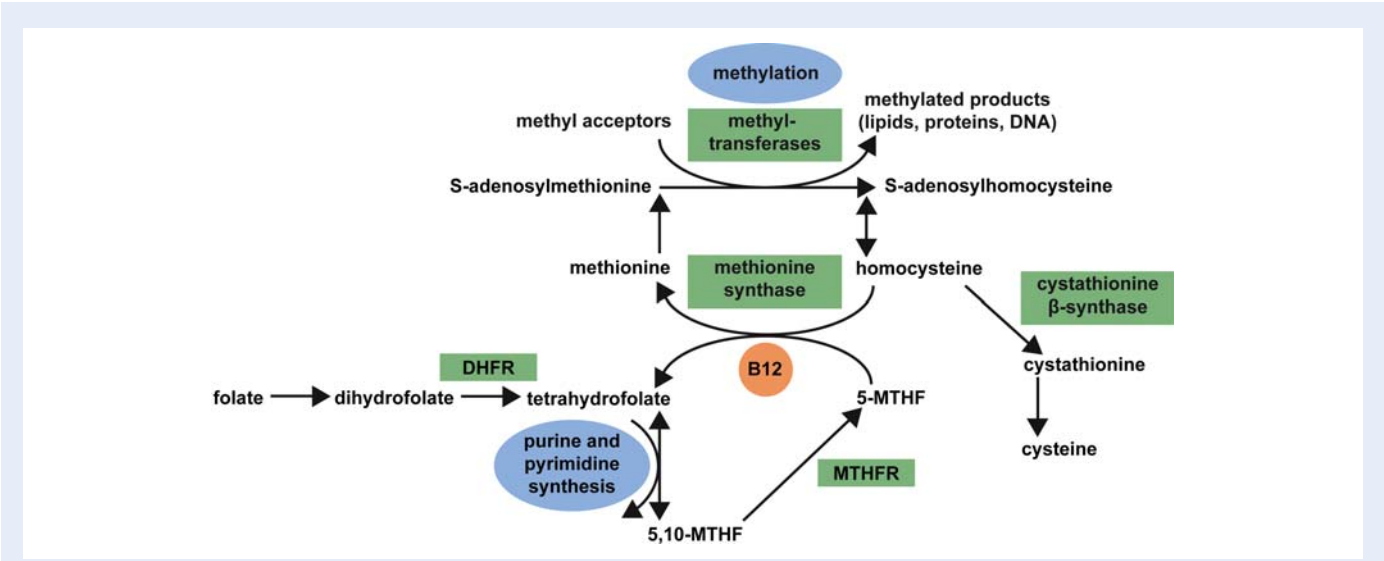


Figure 1 Folate–homocysteine–methionine metabolism. B12, vitamin B₁₂; DHFR, dihydrofolate reductase; MTHF, methyltetrahydrofolate; MTHFR, methyltetrahydrofolate reductase.

Table 1 Medical drugs associated with folate antagonism

Medication	Main indication	Interference with folate metabolism
Carbamazepine	Epilepsy, bipolar disorder	Impairment folate absorption
Cholestyramine	Hypercholesterolemia	Impairment folate and vitamin B ₁₂ absorption
Cyclosporine	Transplants, psoriasis, atopic dermatitis	Possible interference folate dependent remethylation
Lamotrigine	Epilepsy, bipolar disorder	Inhibition DHFR
Metformin	Diabetes	Interference vitamin B ₁₂
Methotrexate	Cancer, some auto-immune diseases (rheumatoid arthritis, psoriasis)	Inhibition DHFR
Nicotinic acid	Hypercholesterolemia	Decrease activity CBS
Phenobarbital	Epilepsy	Impairment folate absorption
Phenytoin	Epilepsy	Impairment folate absorption, decrease activity methionine synthase, possible decrease activity MTHFR
Primidone	Epilepsy	Impairment folate absorption
Pyrimethamine	Malaria	Inhibition DHFR
Sulfasalazine	Inflammatory bowel disease, rheumatoid arthritis	Inhibition DHFR
Triamterene	Hypertension, edema	Inhibition DHFR
Trimethoprim	Urinary tract infection	Inhibition DHFR
Valproic acid	Epilepsy, migraine headache	Antimetabolite of folate

CBS, cystathione β-synthase; DHFR, dihydrofolate reductase; MTHFR, methyltetrahydrofolate reductase.

2006) and methylenetetrahydrofolate dehydrogenase (MTHDF1; Parle-McDermott et al., 2006), may lead to differences in the susceptibility of individuals to folate antagonists.

Experimental studies in a number of animal species demonstrated that folate deficiency causes intrauterine death, growth retardation and various congenital malformations (Jordan et al., 1977; Li et al., 2005). The fact that folic acid supplementation in the periconceptional period decreases the risk of neural tube defects in humans (Lumley et al., 2001) strongly suggests a causative role of folate deficiency in the etiology of these defects. Recently, low blood folate status has

been associated with an increased risk of neural tube defects (Candito et al., 2008; Zhang et al., 2008). Besides folate deficiency, a low maternal vitamin B₁₂ (cyanocobalamin) status has also been shown to be an independent risk factor for neural tube defects (Ray et al., 2007; Molloy et al., 2009). Vitamin B₁₂ is cofactor to methionine synthase, which converts homocysteine into methionine. Therefore, a shortage of vitamin B₁₂ also leads to a distorted folate metabolism.

The exact mechanism by which disturbances of the folate metabolism increase the risk of neural tube defects is unclear. Women

who carry a fetus with a neural tube defect have significantly higher levels of homocysteine in plasma and amniotic fluid than control subjects (Mills *et al.*, 1995; Steegers-Theunissen *et al.*, 1995), which may be caused by folate deficiency. Several hypotheses have been proposed to explain how increased levels of homocysteine, or the accompanying decreased methionine levels, could cause neural tube defects. First, homocysteine itself may be teratogenic during the neurulation process, causing dysmorphogenesis of the neural tube, heart and ventral wall in chick embryos (Rosenquist *et al.*, 1996). In rat and mouse embryos, however, increased homocysteine levels did not cause neural tube defects (van Aerts *et al.*, 1993; Bennett *et al.*, 2006). Therefore, it seems that elevated plasma homocysteine levels itself may not cause neural tube defects, but are a biomarker of disturbances in the methylation cycle which may result in neural tube defects. More likely, intracellular accumulation of homocysteine leads to increased levels of S-adenosylhomocysteine, which is a competitive inhibitor of many methyltransferases, through which gene expression, protein function and the lipid and neurotransmitter metabolisms might be dysregulated (van der Put *et al.*, 2001; Blom *et al.*, 2006). Furthermore, the decreased remethylation of homocysteine to methionine leads to decreased levels of S-adenosylmethionine, which is the most important methyl-group donor in the methylation cycle. As a result, neurulation could be disturbed by inadequate gene and amino acid methylation (van der Put *et al.*, 2001). Methylation steps also play an important role in the metabolism of lipids and neurotransmitters and in detoxification of exogenous substances. This stresses the crucial role of the folate metabolism for normal cellular function, especially during cell division and differentiation. This hypothesis is supported by previous studies showing that methionine is required for normal neural tube closure in rat embryos (Coelho and Klein, 1990; Vanaerts *et al.*, 1994). Disturbances in folate metabolism are also thought to play a role in the etiology of orofacial clefts (Werler *et al.*, 1999; van Rooij *et al.*, 2004; Wilcox *et al.*, 2007), heart anomalies (Czeizel, 1993; Shaw *et al.*, 1995), limb reduction defects (Czeizel, 1993; Shaw *et al.*, 1995; Werler *et al.*, 1999), anal atresia (Myers *et al.*, 2001) and urinary tract anomalies (Czeizel, 1993; Werler *et al.*, 1999) since folic acid supplementation, alone or in multivitamins, seems to have a protective effect on the occurrence of these birth defects, although the evidence is not as strong and consistent as for neural tube defects. Therefore, it seems likely that medications that act as folate antagonists may cause various birth defects through similar mechanisms.

Neural Crest Cell Disruption

The neural crest is an important, pluripotent cell population that originates in the neural folds. The neural crest cells can be divided into two major populations: the cranial and truncal neural crest. During neurulation, the neural crest cells detach from the neural folds and migrate into the embryo to give rise to numerous structures. In the craniofacial region, various cell types and structures, including intramembranous bone, cartilage, nerves and muscles, are derived from the cranial neural crest. The truncal neural crest produces important components of the peripheral nervous system (Larsen, 2001). The cardiac neural crest is a subpopulation of the cranial neural crest, which migrate into the cardiac outflow tract

to mediate septation and into other derivatives of the pharyngeal arches, such as the thymus and the thyroid and parathyroid glands (Kirby and Waldo, 1990). Therefore, neural crest-related cardiovascular malformations include aortic arch anomalies and conotruncal defects (Nishibatake *et al.*, 1987). Membranous ventricular septal defects are also neural crest-related, since the membranous part of the interventricular septum originates from the cardiac neural crest, whereas the muscular part originates from the mesenchyme (Waldo *et al.*, 1998). Non-cardiovascular defects that have been proposed to be neural-crest related are craniofacial malformations (Chai and Maxson, 2006), esophageal atresia (Otten *et al.*, 2000; Morini *et al.*, 2001) and abnormalities of the pharyngeal glands (Bockman and Kirby, 1984).

Proper induction, migration, proliferation and differentiation of neural crest cells are tightly regulated. A variety of molecular signals and receptors are implicated in neural crest cell development. Fibroblast growth factors may be involved in the induction of neural crest cells (LaBonne and Bronner-Fraser, 1998). Integrins, a family of cell surface receptors, play a role in the interaction of neural crest cells with the extracellular matrix (Strachan and Condic, 2008), whereas interactions between neural crest cells are mediated by cadherins (Nakagawa and Takeichi, 1998). It has been suggested that Pax3 is necessary for the fine tuning of the migration process of cardiac neural crest cells (Epstein *et al.*, 2000). Endothelins and their receptors may be required for the migration, differentiation and proliferation of neural crest cells (Clouthier *et al.*, 1998; Yanagisawa *et al.*, 1998). Therefore, drugs that interfere with these molecular pathways, such as bosentan (Clozel *et al.*, 1994), which is indicated for the treatment of pulmonary hypertension and to reduce new digital ulcers associated with systemic sclerosis, may induce neural crest-related malformations. In addition, *in vivo* and *in vitro* experiments suggested that altering levels of folate and/or homocysteine cause abnormalities of cardiac neural crest cell migration, differentiation and cell cycle progression (Stoller and Epstein, 2005), thereby connecting this teratogenic mechanism with folate antagonism. However, one of the most important signaling molecules in neural crest cell development is retinoic acid, the biologically active form of vitamin A. Excesses (Lammer *et al.*, 1985), as well as shortages (Wilson *et al.*, 1953), of retinoic acid seem to cause neural crest-related malformations, indicating that proper retinoid homeostasis is necessary for normal development. Embryonic retinoic acid synthesis and degradation are performed by retinal dehydrogenases and CYP26, respectively (Fujii *et al.*, 1997; Duester, 2000). In addition to retinoids used in the treatment of dermatologic conditions, such as tretinoin, isotretinoin and etretinate, other drugs that inhibit these enzymes may also be involved in disturbances of retinoid homeostasis. It has been suggested that retinoid teratogenicity is mediated by the retinoic acid receptors (RARs) and retinoid X receptors (RXRs; Elmazar *et al.*, 1997). These nuclear ligand-inducible receptors are transcription factors themselves and affect other downstream genes that are important in development (Morris-Kay, 1993). This hypothesis is strengthened by the fact that mice lacking RARs and RXRs show developmental defects similar to those caused by vitamin A deficiency, including neural crest-related malformations (Kastner *et al.*, 1994; Lohnes *et al.*, 1994). Alternatively, increased *Hox* gene expression may underlie the detrimental effects of excess retinoic acid on the development of structures derived from the neural crest (Krumlauf, 1994; Waxman and Yelon, 2009).

Endocrine Disruption: Sex Hormones

Since the 1940s, a number of drugs have been developed to mimic or inhibit the actions of hormones, including diethylstilbestrol (DES), oral contraceptives and hormones used in fertility treatment. These medications and other endocrine disrupting chemicals (EDCs), such as bisphenol A and phthalates, may interfere with the physiologic functions of endogenous hormones by affecting their release, binding or metabolism. Their actions may not only depend upon their affinity or specificity for the estrogen and/or androgen receptors, but also upon their ability to activate or inhibit receptor-mediated actions, which are dependent upon the absorption, distribution, metabolism and excretion (ADME) of these molecules as well. The actions of EDCs *in utero* have been of concern because of their possible impact on the developing reproductive systems, especially since treatment of pregnant women with the synthetic estrogen DES led to an increased risk of vaginal adenocarcinoma in their daughters (Herbst et al., 1971). Since human effects were identified first, animal studies have been conducted to confirm these clinical observations and to investigate the differences between synthetic and natural estrogen actions on the embryo or fetus (McLachlan, 1981; Henry et al., 1984). It is well known that human sex hormone-binding globulin has a substantially higher affinity for estradiol than for DES or other synthetic hormones (Hodgert Jury et al., 2000), which suggests that DES may be more readily available to cross the placenta. DES is also metabolized to reactive intermediates which covalently bind (Metzler, 1981; Miller et al., 1982), whereas estradiol is not metabolized to similar reactive intermediates (Klopper, 1980; Slikker et al., 1982). In addition, α -fetoprotein binds estradiol but not DES (Sheehan and Young, 1979). So besides the capability of the placenta to reduce the transfer of estradiol, plasma binding and metabolism of this endogenous hormone to less active estrogens may be important defense mechanisms for the fetus to reduce the actions of estradiol, which are apparently not available for the synthetic estrogen DES.

Besides an increase in the risk of vaginal adenocarcinoma in daughters, prenatal exposure to DES has also been associated with an increase in reproductive disorders in sons (Giusti et al., 1995) and grandsons (Klip et al., 2002; Brouwers et al., 2006). In male animals, prenatal exposure to EDCs with estrogenic or anti-androgenic properties have been shown to cause hypospadias and cryptorchidism (McMahon et al., 1995; Kim et al., 2004; Christiansen et al., 2008). In addition to drugs that influence endocrine homeostasis as their primary mechanism of action, coatings for oral medications, such as mesalamine and omeprazole, may be a source of EDC exposure (Hernández-Díaz et al., 2009). These enteric coatings contain phthalates, which may affect human male reproductive development due to their anti-androgenic properties (Swan et al., 2005). Additionally, other preparations may contain phthalates as plasticizers (Hauser et al., 2004), but it should be noted that phthalates do not bio-accumulate and are excreted rapidly in contrast to some other EDCs. The susceptibility to EDCs may also vary greatly between individuals due to genetic factors (Giwerzman et al., 2007). Therefore, it is questionable whether the levels of phthalates in medications in particular are high enough to produce male reproductive tract anomalies in humans. In epidemiologic studies, omeprazole and mesalamine have

not been associated with an increased risk of major birth defects (Diav-Citrin et al., 1998; Gill et al., 2009).

Male development is more susceptible to endocrine disruption than female development because of its hormone dependence (Sharpe, 2006). However, since synthetic hormones and EDCs may affect endocrine homeostasis in multiple ways, the underlying teratogenic mechanisms are often difficult to unravel. Because of considerable species differences and markedly different estrogen levels in normal human pregnancy compared with normal rodent pregnancy, it is debatable whether certain mechanisms also apply to humans. Male sexual differentiation generally depends on a balanced androgen/estrogen ratio. In mice, estrogens impair fetal Leydig cell development, and, as a consequence, testosterone production is decreased (Delbès et al., 2005). Phthalates that induce male reproductive disorders in rats mainly do so through inhibition of steroidogenesis by the fetal testis (Parks et al., 2000; Mylchreest et al., 2002), but this does not occur *in vitro* with human fetal Leydig cells (Lambrot et al., 2009). Testosterone secretion is responsible for most of the masculinization process, including the development of the male reproductive tract and external genitalia. Therefore, compromised testosterone production may result in hypospadias. In addition, estrogen exposure also suppresses the production of insulin-like factor 3 by fetal Leydig cells (Emmen et al., 2000). This peptide regulates the growth of the gubernaculum (Adham and AgoulNIK, 2004), which is responsible for testicular descent (Hutson et al., 1997). In humans, a deficiency in androgen production or action seems far more important than estrogen exposure in the etiology of cryptorchidism, since the inhibitory effects of estrogens on testicular steroidogenesis and testicular descent are only mediated through estrogen receptor α in mice (Cederroth et al., 2007), which is not present in the human fetal testes (Gaskell et al., 2003). However, this receptor is expressed and functional in human fetal penile tissue (Crescioli et al., 2003), so a role of estrogen exposure in the induction of hypospadias cannot be excluded. Epidemiologic studies could not confirm this, since prenatal estrogen exposure, including pharmaceutical estrogens, does not seem to be related to hypospadias and cryptorchidism (Storgaard et al., 2006; Martin et al., 2008).

Alternative mechanisms by which EDCs could cause male reproductive disorders have also been suggested. These mechanisms include disruption of the androgen signaling pathway (e.g. suppression of androgen receptor expression), resistance to anti-Müllerian hormone (AMH) and inhibition of enzymes involved in the inactivation of sex steroids. However, involvement of these mechanisms in endocrine disruption seems unlikely for various reasons. Although it has been shown that fetal exposure to chemicals that alter the androgen signaling pathway can induce hypospadias and cryptorchidism in rats (Rider et al., 2008), the dose needed to induce these effects is very high, which makes this mode for EDC-induced teratogenesis doubtful. AMH is primarily responsible for the regression of the Müllerian tract in male embryos (Josso et al., 2001) and may play a role in testicular descent (Hutson et al., 1997). So far, however, no compounds have been identified that affect the production or action of AMH (Sharpe, 2006). The same argument can be applied to the inhibition of estrogen sulfotransferases (and probably other enzymes involved in sex steroid metabolism), which increases cellular estradiol bioavailability. Metabolites of various polycyclic aromatic hydrocarbons inhibit

this enzyme (Kester *et al.*, 2000), but pharmacological compounds with a similar mechanism of action have not been identified yet.

Oxidative Stress

In vivo, several drugs, known as redox cycling agents and used in the treatment of, among others, epilepsy, cardiac arrhythmias and cancer, undergo single electron reduction reactions yielding radical species (Kappus, 1986). In redox cycling reactions which involve oxygen reactive oxygen species (ROS), such as hydrogen oxide, alkyl peroxides and various radicals (e.g. hydroxyl and superoxide), are generated (Kovacic and Somanathan, 2006). The creation of ROS is induced by internal and external agents, such as phagocytes, enzymes like cytochrome P450 mono-oxygenases (CYP), irradiation and exogenous chemicals. In much the same manner, the generation of ROS can be decreased or reversed by various enzymes, e.g. superoxide dismutase, catalase and glutathione reductase, and by antioxidants (Kovacic and Jacintho, 2001). Endogenous ROS serve as a second messenger in signal transduction (Hansen, 2006) and are thought to be important in ion transport, immunological host defense, transcription and apoptosis of unwanted cells (Lander, 1997; Dennerly, 2007). However, ROS can also be harmful by binding covalently or irreversibly to cellular macromolecules. Oxidative stress, an imbalance between ROS generation and antioxidant defense mechanisms of a cell or tissue, causes irreversible oxidation of DNA, proteins and lipids, leading to inactivation of many enzymes and cell death (Fig. 2). In addition to damaging cellular macromolecules, oxidative stress may affect gene expression by interfering with the activity of redox-sensitive transcription factors and signal transduction by oxidizing thiols (Sahambi and Hales, 2006). During the prenatal period, this may result in birth defects and growth retardation, and in severe cases in *in-utero* death (Trocino *et al.*, 1995; Wells *et al.*, 1997; Hansen, 2006).

The developing embryo is especially susceptible to high levels of ROS because of its weak antioxidant defense, in particular in the early stages of organogenesis (Zaken *et al.*, 2000), although placental enzymes play a role in protecting the fetus against oxidative stress

(Foster *et al.*, 2008). Oxidative stress is postulated to be involved in the pathogenesis of a wide spectrum of birth defects, including skeletal malformations (Sahambi and Hales, 2006; Yan and Hales, 2006), limb defects (Wellfelt *et al.*, 1999; Fantel and Person, 2002), neural tube defects (Ishibashi *et al.*, 1997; Ryu *et al.*, 2007), cleft lip/palate (Wellfelt *et al.*, 1999; Winn and Wells, 1999) and cardiovascular defects (Wellfelt *et al.*, 1999). Several drugs are known to induce oxidative stress, which is suspected to be their main teratogenic mechanism. Among these drugs are thalidomide (Hansen and Harris, 2004), phenytoin (Liu and Wells, 1994; Winn and Wells, 1999), valproic acid (Defoort *et al.*, 2006), class III antiarrhythmic drugs (Wellfelt *et al.*, 1999; Danielsson *et al.*, 2003), iron supplements (Scholl, 2005) and various chemotherapeutic drugs (Kovacic and Jacintho, 2001).

However, it is important to notice that ROS are intermediary compounds with unpaired electrons and, as a consequence, have a very short lifetime ranging from nanoseconds to milliseconds. Therefore, ROS are generally too unstable to be transferred from the mother to the developing embryo or fetus. Whenever ROS are increased in embryos, it is the result of embryonic metabolic changes rather than exposure to ROS of maternal origin (Ornøy, 2007). Increases in embryonic ROS may be caused by increased enzymatic bioactivation of proteratogens, including bioactivation of the aforementioned drugs. However, most isoforms of the CYP family, which catalyze the bioactivation of many compounds after birth, are expressed at relatively low levels during the embryonic period. Only some isoforms are expressed at levels that could be significant in teratogenesis (Juchau *et al.*, 1992; Wells and Winn, 1996). In contrast, the prostaglandin H synthases (PHSs) have a relatively high expression during the embryonic and fetal period compared with expression after birth (Winn and Wells, 1997; Parman and Wells, 2002). The peroxidase component of this enzyme can bioactivate exogenous substances, including phenytoin and related teratogens (Parman *et al.*, 1998), to toxic reactive intermediates that initiate ROS formation (Eling *et al.*, 1990). There is evidence that lipoxygenases (LPOs), which oxidize proteratogens yielding free radical intermediates, are substantially expressed in embryonic tissues as well (Yu and Wells, 1995). As a result, it is assumed that

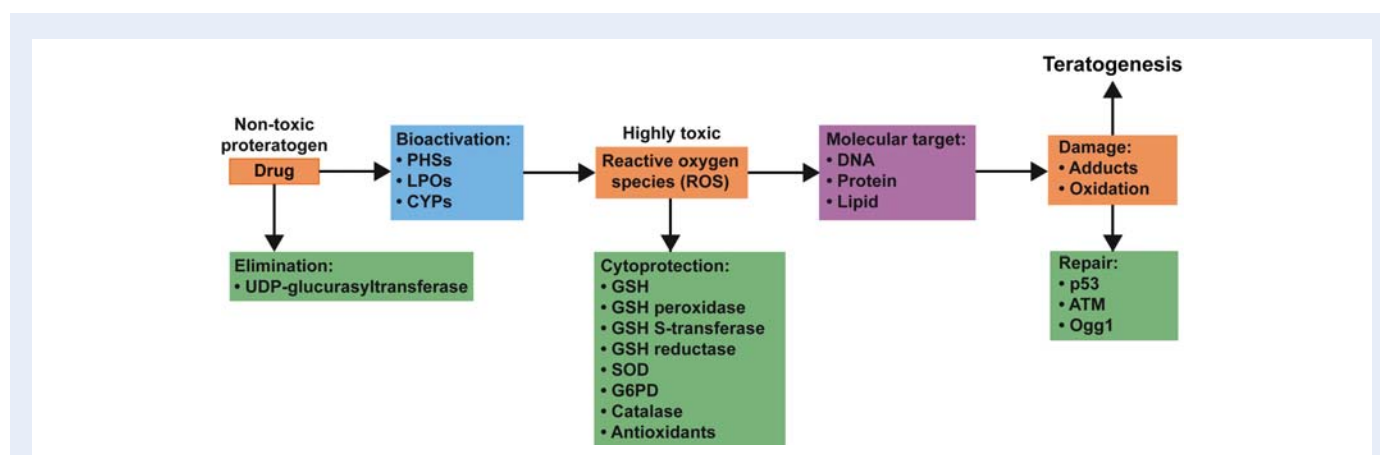


Figure 2 Molecular and biochemical determinants of oxidative stress teratogenesis. ATM, ataxia telangiectasia mutated; CYP, cytochrome P450; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; LPO, lipoxygenase; Ogg1, oxoguanine glycosylase 1; PHS, prostaglandin H synthase; SOD, superoxide dismutase; UDP, uridine diphosphate. Modified from Winn and Wells (1995) with kind permission from Wiley-Blackwell.

bioactivation of proteratogens by embryonic PHSs and LPOs is necessary for the formation of ROS and subsequent macromolecule damage in the developing embryo (Wells et al., 1997). Additionally, embryonic ROS formation and subsequent oxidative stress may be induced by hypoxia. It is well known from adult cases of cardiovascular diseases (Madamanchi et al., 2005) that ROS are extensively formed during reperfusion of ischemic tissues, while there is considerable evidence that hypoxia followed by reperfusion is teratogenic in animal studies (Wellfelt et al., 1999). Besides embryonic ROS generation, maternal determinants are thought to play an indirect role in ROS-mediated teratogenesis. Embryonic exposure to proteratogens is altered by maternal pathways that eliminate these compounds or their metabolites before they can cross the placenta. Deficiencies in those pathways increase the maternal plasma concentration of proteratogens and therefore the amount that reaches the embryo. Furthermore, maternal production of factors that interfere with embryonic ROS-mediated signal transduction or alter embryonic determinants of oxidative stress may also contribute to the risk of teratogenicity (Wells et al., 2005).

Vascular Disruption

Vascular disruption defects are structural birth defects resulting from interference with or extrinsic breakdown of an originally normal prenatal development of the arteries, veins and capillaries (vasculature) (Spranger et al., 1982; Gilbert-Barness and Van Allen, 2007). Traditionally, it has been stressed that a teratogen exerts its influence on the fetus during the first 3 months of development. Prenatal exposure to agents which can induce vascular disruption, however, can also induce damage later in pregnancy to structures that were initially formed normally. After birth it may be impossible to determine whether a certain structural anomaly, such as a limb defect, is the result of an intrinsically abnormal developmental process, vascular disturbances or, for example, amniotic banding.

Vascular disruption refers to disturbances in the blood circulation in the uterine-placental unit, the placental-fetal unit or the fetus itself. These disturbances include hyperperfusion, hypoperfusion, hypoxia and obstruction. They may be caused by acute or chronic decreases in uterine blood flow, vascular infections or an abnormal anatomy in the uterine-placental unit. Factors such as placental insufficiency, amnion rupture and umbilical cord obstruction may cause failures in the vascular supply in the placental-fetal unit. In the fetus, disruption of newly formed vessels, external compression, embolic events, premature regression of embryonic vessels, occlusion with venous engorgement and abnormal regulation of vessel formation lead to vascular disruption (Van Allen, 1992). Vasoconstriction of maternal and fetal vessels, hypoperfusion and obstruction may cause a reduced supply of nutrients to the embryonic tissues, which can affect development and growth of embryonic structures or result in tissue loss. The latter may result in a phenotype similar to a primary malformation (Hootnick et al., 1980). Furthermore, these disturbances may create a state of hypoxia, which is involved in the formation of ROS and oxidative stress (Ornoy, 2007).

Exposure to vasoactive substances in pregnancy, especially to those with vasoconstrictive effects, have been hypothesized to play a causal role in vascular disruption defects. These teratogens could decrease placental or fetal blood flow or affect the development of blood

vessels, thereby changing the structure and/or anatomy of the vasculature (Gilbert-Barness and Van Allen, 2007). In epidemiologic studies, vasoactive therapeutic drugs that have reported associations with the vascular disruption defects described below include misoprostol (Orioli and Castilla, 2000; Vargas et al., 2000), aspirin (Kozer et al., 2002; Werler et al., 2002), ergotamine (Raymond, 1995; Smets et al., 2004) and pseudoephedrine (Werler et al., 2002; Werler et al., 2004). However, all drugs with vasoconstrictive or vasodilating effects may have the potential to cause birth defects due to vascular disruption.

The types of structural anomalies that may be caused by vascular disruption are determined by the timing during gestation, the location and severity of tissue damage and the possible presence of secondary adhesion of necrotic tissue with adjacent organs or the amnion (Gilbert-Barness and Van Allen, 2007). During embryogenesis, vascular disruption results in aberrant differentiation and distortion of contiguous tissues, loss of tissue and incomplete development of structures within the same or a secondary embryonic developmental field. Anomalies resulting from vascular disruption during the fetal period are usually limited to the areas with disturbed blood supply, to which the peripheral vasculature is most susceptible (Van Allen, 1992). Therefore, the majority of defects caused by tissue damage through vascular disruption occur in structures supplied by the most peripheral vasculature, such as the distal limbs and the embryonic intestine (Jones, 1991; Los et al., 1999). Birth defects that were attributed to vascular disruption include terminal limb reductions (Kino, 1975; Hoyme et al., 1982), hydranencephaly/porencephaly (Hoyme et al., 1981a; Mittelbronn et al., 2006), gastroschisis (Hoyme et al., 1981b; Komuro et al., 2003), small intestinal atresia (Louw and Barnard, 1955; Cragan et al., 1994) and Poland anomaly (Shalev and Hall, 2003; Puvabanditsin et al., 2005). However, there are no known experimental models for the complete range of birth defects caused by vascular disruption. The majority of evidence in support of this mechanism comes from case reports with suspected vascular events such as occlusion, emboli, amnion rupture and twin placental vessel anastomoses (Gilbert-Barness and Van Allen, 2007).

Specific Receptor- or Enzyme-mediated Teratogenesis

Many medical drugs act on a specific receptor or enzyme in the human body, leading to a particular mechanism of action. Below we describe the possible effects of inhibition or stimulation of some of these specific receptors and enzymes on fetal development.

Angiotensin-converting enzyme and angiotensin II receptors

The renin-angiotensin system (Fig. 3) is generally described as a hormonal system that plays an important role in the regulation of blood pressure and in the homeostasis of extracellular fluid volume. The main effector hormone of this system is angiotensin II (AT II), which elevates blood pressure by acting directly on vascular smooth muscle cells to cause vasoconstriction. The components of the renin-angiotensin system are present in the human fetus, although their distribution varies compared with that in adults (Schütz et al.,

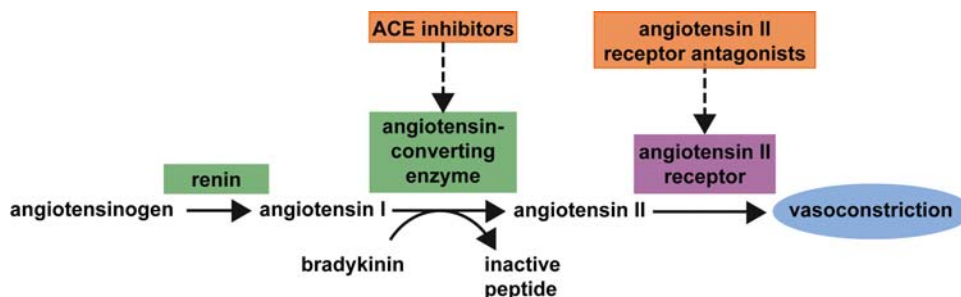


Figure 3 The renin–angiotensin system. ACE, angiotensin-converting enzyme.

1996). Two types of commonly used antihypertensive drugs, the angiotensin-converting enzyme (ACE) inhibitors and the AT II receptor antagonists, may disrupt the fetal renin–angiotensin system and thereby impair fetal development. In contrast to other antihypertensive drugs, ACE inhibitors and AT II receptor antagonists also influence renal function (Jackson and Garrison, 1996). Therefore, their effects are not exclusively produced through fetal hypotension and vascular disruption. The decrease in fetal renal vascular tone may contribute to a human malformation syndrome that is typical for exposure to ACE inhibitors during the second and third trimesters of pregnancy, characterized by renal tubular dysgenesis and oligohydramnios, their sequelae, including limb contractures and pulmonary hypoplasia, and hypocalvaria (Pryde *et al.*, 1993; Shotan *et al.*, 1994). Although the two AT II receptor subtypes, AT₁ and AT₂, are expressed in early development (Schütz *et al.*, 1996), the developmental effects of ACE inhibitors during the first trimester are controversial. However, a recent study showed an increased risk of cardiovascular and central nervous system malformations (Cooper *et al.*, 2006). The effects of the less often studied AT II receptor inhibitors are considered to be similar to those of ACE inhibitors.

Hydroxymethylglutaryl-coenzyme A reductase

The mevalonate pathway is a complex pathway with cholesterol as an essential product. In embryonic tissues, cholesterol is needed for normal growth patterns, signaling domains in plasma membranes, synthesis of steroid hormones and activation of Hedgehog morphogens (Carr *et al.*, 1980; Kelley and Herman, 2001). Since Hedgehog proteins act as key regulators of embryonic growth, patterning and morphogenesis of many structures, down-regulation of the synthesis of these proteins may lead to birth defects (Helms *et al.*, 1997; Gofflot *et al.*, 2003). Statins inhibit hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway which converts HMG-CoA to mevalonic acid. Therefore, inhibition of this pathway by statins may lead to a wide range of defects. However, epidemiologic studies with appropriate control populations to confirm a statin syndrome in humans have not been performed yet due to the low frequency of statin use among pregnant women. Although a recurrent pattern of structural defects has been described (Edison and Muenke, 2004), a recent study could not confirm this hypothesized pattern (Petersen *et al.*, 2008).

Histone deacetylase

Histone deacetylases (HDACs) are present in most organisms, in which their best known function is the deacetylation of histones. These are crucial in a number of cellular functions, including the regulation of gene expression by chromatin remodelling. HDACs deacetylate lysine residues on histone tails and condensate chromatin, resulting in limited access of transcriptional activators to the DNA (Johnstone, 2002). Therefore, inhibition of HDACs may result in interruption of cell proliferation, differentiation and apoptosis (Marks *et al.*, 2000), which has been shown in cultured tumor cells (Medina *et al.*, 1997; Glick *et al.*, 1999). Although normal cells seem to be relatively resistant to HDAC inhibitors (Qiu *et al.*, 2000; Burgess *et al.*, 2004), HDAC activity is crucial for embryonic development as is shown by the HDAC1 knockout mice, which die early in development due to growth retardation and proliferation defects (Lagger *et al.*, 2002). Not much has been published on the effects of HDAC inhibition in the pathogenesis of human birth defects, but animal studies show that it might lead to axial skeletal malformations (Menegola *et al.*, 2005; Di Renzo *et al.*, 2007) and neural tube defects (Eikel *et al.*, 2006). Drugs that inhibit HDACs include valproic acid (Göttlicher *et al.*, 2001; Phiel *et al.*, 2001), trichostatin A (Yoshida *et al.*, 1990) and salicylates (Di Renzo *et al.*, 2008). Furthermore, boric acid, an inactive ingredient used in pharmaceutical preparations and as an antibacterial product in non-prescription products, may induce hyperacetylation in somites (Di Renzo *et al.*, 2007).

Cyclooxygenase-I

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for their analgesic, antipyretic and anti-inflammatory effects induced by acting as an inhibitor of cyclooxygenases (COXs), which catalyze the conversion of arachidonic acid to prostaglandins. Two distinct isoforms have been identified, COX-1 and COX-2. The constitutive form, COX-1, is expressed in most tissues, where it produces prostaglandins that are necessary for various physiologic processes, such as blood pressure regulation and platelet aggregation. COX-2 expression, on the other hand, is induced by inflammatory mediators, producing prostaglandins which are important in inflammation (Vane *et al.*, 1998). The anti-inflammatory properties of NSAIDs are due to the inhibition of COX-2, whereas the adverse effects of non-selective NSAIDs, which inhibit both COX isoforms, are the result of COX-1 inhibition (Vane *et al.*, 1998). COX-1 inhibition may be involved in the induction

of cardiac, midline and diaphragm defects by non-selective NSAIDs, since these defects were associated with exposure to drugs with a relatively high COX-1/COX-2 ratio in rats and rabbits (Cappon et al., 2003). Furthermore, COX-2 is not expressed during embryogenesis in rats (Stanfield et al., 2003; Streck et al., 2003), which strongly suggests that COX-2 does not play a role in NSAID-induced teratogenicity noted in this species. Acetylsalicylic acid (aspirin), the only NSAID that irreversibly inhibits COX by acetylation (Vane et al., 1998), seems to be associated with a higher incidence of malformations than other NSAIDs in animal studies (Cook et al., 2003). Initially, first trimester exposure to NSAIDs did not seem to be associated with birth defects in humans (Nielsen et al., 2001; Cleves et al., 2004), but recent epidemiologic studies indicate an increased risk of orofacial clefts and cardiovascular defects, especially cardiac septal defects (Ericson and Källén, 2001; Källén and Otterblad Olausson, 2003; Ofori et al., 2006).

N-methyl-D-aspartate receptors

In the developing brain, N-methyl-D-aspartate (NMDA) receptors appear to play an important role in neuronal migration and in the formation and elimination of synapses (Komuro and Rakic, 1993). Blockade of the NMDA receptor in studies using NMDA receptor antagonists or knockout mice affect neuronal development (Komuro and Rakic, 1993; Elberger and Deng, 2003), which may result in structural abnormalities of the brain due to errors in migration of neuronal and glial elements (Clarren et al., 1978). Rats are most vulnerable to the effects of NMDA receptor antagonists in the first week after birth (Ikonomidou et al., 1999), during which the expression of NMDA receptors peaks (Monyer et al., 1994) and the brain growth spurt occurs (Dobbing and Sands, 1971). Since the expression of NMDA in humans peaks in weeks 20–22 of gestation (Lee and Choi, 1992), during which the brain growth spurt starts, and continues throughout the third trimester and postnatally (Dobbing and Sands, 1973), it has been hypothesized that humans might be susceptible to the effects of NMDA receptor antagonists from 20 weeks of gestation onward (Ikonomidou et al., 1999). Therefore, it may be concluded that exposure to NMDA receptor antagonists, such as amantadine (Kornhuber et al., 1991), dextromethorphan (Wong et al., 1988) and ketamine (Anis et al., 1983), could result in minor malformations of the brain. Controversial is the suggested role of NMDA receptor antagonists in the induction of neural tube and neural crest defects, as shown by Andaloro et al. (1998) using chick embryos. These results could not be replicated in mice (Bennett et al., 2006) and the widely used drug dextromethorphan does not seem to be associated with congenital defects in humans (Briggs et al., 2008). Although NMDA receptors are being expressed in the human spinal cord during the first trimester (Åkesson et al., 2000), inhibition of these receptors does not appear to play a role in the induction of neural tube and neural crest defects. Therefore, it is questionable whether this mechanism produces major structural birth defects in humans.

5-Hydroxytryptamine receptors and transporters

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter, which is derived from the maternal circulation and transported

to the embryo (Yavarone et al., 1993a). It is involved in a wide range of processes during development, including morphogenesis of craniofacial structures (Shuey et al., 1993), cranial neural crest migration (Moi-seiwitsch and Lauder, 1995) and cell proliferation (Lauder, 1993). The effects of 5-HT appear to be mediated by 5-HT receptors (Choi et al., 1997), G-protein-linked transmembrane receptors with the exception of the 5-HT₃ receptor, which is a ligand-gated ion channel. At least some of the 5-HT receptor subtypes are expressed in mice embryos, and these are shown to be involved in the morphogenesis of various embryonic tissues (Lauder et al., 2000; Nebigil et al., 2001). Therefore, increased stimulation or suppression of 5-HT receptors by agonists and antagonists may cause birth defects. Drugs known to be agonists of some 5-HT receptor subtypes include sumatriptan (Scott, 1994) and buspirone (Tunnicliff, 1991), whereas, among others, risperidone (Schotte et al., 1996), granisetron (Blower, 2003) and quetiapine (Meltzer et al., 2003) antagonize some 5-HT receptor subtypes. Furthermore, the actions of 5-HT are terminated by the uptake of the neurotransmitter by serotonin transporters, implying that prenatal exposure to selective serotonin-reuptake inhibitors (SSRIs) may also cause birth defects. This class of antidepressants, which includes fluoxetine, paroxetine and sertraline, has been shown to cause craniofacial malformations in mice (Shuey et al., 1992). 5-HT also seems to be involved in cardiac morphogenesis (Yavarone et al., 1993b; Sari and Zhou, 2003), indicating that blockade of 5-HT uptake might produce cardiovascular malformations as well. In humans, however, the risk of birth defects associated with SSRIs as a group appears to be small (Kulin et al., 1998; Alwan et al., 2007; Louik et al., 2007), although recent reports suggest an association between paroxetine use and birth defects (Bérard et al., 2007; Källén and Otterblad Olausson, 2007), but this has been refuted by others (Einarson et al., 2008). An association between first-trimester exposure to fluoxetine and cardiovascular anomalies has been suggested as well (Diav-Citrin et al., 2008). Therefore, it may be hypothesized that individual SSRIs may have different effects on the developing embryo. Due to the inconsistencies in the results of epidemiologic studies, one may suspect that other issues also play a role in this possible association, including disease status of the mother and other confounding factors, such as detection bias and use of concomitant medications.

γ-Aminobutyric acid receptors

In vertebrates, γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter, which binds to specific transmembrane GABA receptors. Extraneuronal GABA-ergic systems are thought to be present in other tissues as well, including the testis (Tillakaratne et al., 1992), oviduct and ovary (Erdö et al., 1989; Tillakaratne et al., 1992) and pancreas (Baekkeskov et al., 1990), where GABA is hypothesized to play a morphogenetic role during embryonic development (Varju et al., 2001). The extraneuronal GABA-ergic system also seems to play an important role in the normal development of the palate (Hagiwara et al., 2003), but the exact function of this system in non-neural tissues is still unknown. The major groups of drugs that exert their pharmacologic actions through GABA receptors are benzodiazepines, which enhance the effects of GABA (Haefely, 1984). Although these drugs are commonly used during pregnancy and neonatal complications such as the 'floppy infant syndrome' and

the 'withdrawal syndrome' have frequently been observed data on the teratogenicity of benzodiazepines are scarce and inconsistent. In some epidemiologic studies, use of benzodiazepines in the first trimester has been associated with orofacial clefts (Dolovich *et al.*, 1998), cardiovascular malformations (Czeizel *et al.*, 2004) and gastrointestinal tract atresia (Norstedt Wikner *et al.*, 2007), but other studies did not find an association with birth defects (Rosenberg *et al.*, 1983; Ornoy *et al.*, 1998; Lin *et al.*, 2004).

Carbonic anhydrase

Carbonic anhydrases are metalloenzymes that catalyze the reversible hydration of CO₂ into the bicarbonate ion and protons. This reaction is involved in many biological processes, including pH homeostasis, respiration, biosynthetic reactions and bone resorption (Maren, 1967; Sly and Hu, 1995). Several cytoplasmic and membrane-bound carbonic anhydrase isoenzymes are expressed in various tissues in developing human and mouse embryos (Jeffery *et al.*, 1980; Lönnerholm and Wistrand, 1983; Kallio *et al.*, 2006), and inhibitors of carbonic anhydrase, such as acetazolamide, which is used in the treatment of epilepsy, altitude sickness, edema and sleep apnea, have been associated with birth defects, especially limb deformities (Layton and Hallesy, 1965; Scott *et al.*, 1990). A reduction in embryonic intracellular pH is thought to be the teratogenic mechanism of carbonic anhydrase inhibitors (Scott *et al.*, 1990). Intracellular pH has been shown to control or to be associated with various cellular functions, including protein synthesis, proliferation and glycolysis (Madshus, 1988). Interference with these processes may result in abnormal development, but evidence of the existence of this mechanism in humans is lacking.

Summary

From the literature, we identified six principal teratogenic mechanisms associated with medical drug use. Beside the fact that almost all medical drugs classified by Schwarz *et al.* (2007) as U.S. FDA class X are associated with at least one of these mechanisms, various other prescription and over the counter drugs may produce teratogenic effects through these mechanisms. Increased risks for specific birth defects have been observed for some medical drugs after use in human pregnancy, which strengthens the evidence in favor of the associated teratogenic mechanisms. However, since the possibilities to conduct experiments during human pregnancy are very limited, the major part of the evidence in support of various mechanisms described above was derived from animal studies, in which the dosages administered were often far above the therapeutic dosage schedules used in humans. Therefore, we cannot be sure that these mechanisms also apply to humans. In addition, some mechanisms share similar pathways and some drugs may be involved in multiple mechanisms, e.g. valproic acid. Nevertheless, the identification of teratogenic mechanisms are critical for research purposes, in particular for observational studies, in which specific medications with a similar teratogenic mechanism might be combined to increase study power. It may have implications for drug development and for prescribing multiple drugs to women of reproductive age as well, especially since combinations of seemingly unrelated drugs may produce specific teratogenic mechanisms, which may strongly increase the risk of birth

defects. Given that discontinuing a certain medication may pose even a higher risk for severe complications than continuing with the use of a possible teratogen, the benefits for the mother should always be balanced against the risks for the (unborn) child when prescribing drug treatment to pregnant women.

Authors' Roles

M.v.G.: lead author, responsible for study design, literature search, data interpretation, preparation of draft manuscript. I.v.R.: study design, data interpretation, critical review manuscript. R.M.: agreed study design, data interpretation, critical review manuscript. G.Z.: agreed study design, data interpretation, critical review manuscript. L.d.J.v.d.B.: agreed study design, data interpretation, critical review manuscript. N.R.: study design, supervised literature search, data interpretation, critical review manuscript.

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